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MATURATION OF THE ADRENAL MEDULLA: CATECHOLAMINE STORES IN NORMAL AND HYPERTENSIVE RATS

The first progress report from this project detailed the biochemical maturation of the adrenal medulla in normotensive Wistar rats (NWR) and in the second report these changes were identified on the molecular level and reduced to a set of mathematical parameters describing the development process. Additionally, studies were commenced utilizing similar techniques to evaluate adrenal catecholamine synthesis, uptake, storage and release in adult spontaneously hypertensive rats (SHR). To determine whether increased sympatho-adrenal activity plays a role in the development of hypertension, the studies concentrated the properties of the adrenomedullary storage vesicles.

The uptake of ^{14}C-epinephrine per 100 μg of endogenous catecholamines in isolated SHR vesicles was higher than in NWR while the uptake of ^{3}H-metaraminol was the same as in NWR; thus, SHR vesicles exhibited a higher preference for epinephrine vs. metaraminol compared to NWR. The difference in uptake was due to a lower Km for epinephrine in SHR. The storage of amines was the same in SHR and NWR, as demonstrated by measurements of catecholamine to ATP ratios in purified vesicles, and effluxes from the vesicles of endogenous and newly-incorporated amines. The ratio of catecholamines to dopamine β-hydroxylase (DBH, a marker for storage vesicles) was higher in SHR for three reasons: (1) there were fewer vesicles per gland; (2) there was less DBH per vesicle, indicated by an increased precursor/product ratio ; and (3) there was a higher catecholamine content per vesicle, as shown by an increase in the ratio of heavy to light vesicles on discontinuous sucrose density gradients. SHR adrenals were depleted of catecholamines after insulin administration to a greater extent than were NWR adrenals, and both SHR and NWR exhibited induction of tyrosine hydroxylase and dopamine β-hydroxylase after insulin. None of these findings is consistent with the view that sympatho-adrenal hyperactivity occurs in the SHR; the data suggest that hypoactivity occurs, perhaps secondarily to the hypertension. However, the sympatho-adrenal axis of the SHR appears to be set on a "hair trigger" such that stimuli which usually cause a small or moderate discharge result in a much larger discharge in the SHR. This may be of some significance in evaluating the effects of chronic, low level stress in the SHR, such as that produced by low doses of nicotine (see discussion of future experiments).

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Our latest studies have tested the effects of the antihypertensive drug, reserpine, in SHR and NWR. Reserpine elicits a triphasic response -- initially there is a reflex stimulation of the sympatho-adrenal axis similar to the effect of insulin. This is followed by catecholamine depletion from reserpine blockade of the vesicular uptake mechanism; thirdly, there is a late increase in stimulation resulting from the hypotensive effect of the drug. We have found that the SHR is more susceptible to the initial and late stimulatory phases of reserpine action, as would be expected from the hypersecretion obtained from insulin. The differences between NWR and SHR in these phases can be eliminated by administration of the ganglion blocker, chlorisondamine. When this is done, SHR appear to be less sensitive to the depletion effect of reserpine, probably as a result of the alteration in the Km for uptake of epinephrine. If human essential hypertension is in fact similar to the SHR, these data might explain the development of refractoriness to antihypertensive medications which act on the level of the storage vesicle (reserpine, aldomet).

One important question which was unresolved was whether the hypersecretion on stimulation of the SHR adrenal resulted from a central effect (increased frequency of action potentials coming down the splanchnic nerve) or whether the gland hyperresponds to the same number of stimuli. To test this hypothesis, direct secretion was evoked by nicotine administration. Under these conditions, an equivalent secretory response was obtained in SHR and NWR, indicating that the defect is central in origin.

Over the next 6 months we expect to extend these findings to cover other important antihypertensive medications (aldomet, DBH inhibitors, α-methyl-p-tyrosine, 6-hydroxydopamine) and to commence further studies on the acute and chronic effects of low and high doses of nicotine, which is reputed to cause persistent alterations in autonomic control of blood pressure; our preliminary work on acute nicotine administration suggests that the SHR is not hypersensitive to the drug, but other investigators have shown that alterations in catecholamine turnovers usually appear only after chronic administration. Obviously, chronic studies are required to see if differences appear in the SHR vs. the NWR.

In evaluating these changes, it is apparent that one must differentiate between those which are involved in the hypertensive process and those which are unrelated ancillary genetic alterations. For this reason, we originally intended immediately to examine the development of amine stores and hypertension in the maturing SHR, to see whether sympatho-adrenal changes precede or follow the development of high blood pressure. Because of delays in obtaining a reliable source of pregnant SHR (there is only one commercial supplier), these studies will be delayed until this winter; Carworth Farms has assured us the animals will be available at that time.

Publications from this project:

1. Maturation of the adrenal medulla. I. Uptake and storage of amines in isolated vesicles of the rat. T.A. Slotkin, Biochem. Pharmacol. 22:2023-2032 (1973).

2. Maturation of the adrenal medulla. II. Content and properties of catecholamine storage vesicles of the rat. T.A. Slotkin, Biochem. Pharmacol. 22:2033-2044 (1973).

3. Binding of amines to purified bovine adrenal medullary storage vesicle membranes. T.A. Slotkin and N. Kirshner, Biochem. Pharmacol. 22:2492-2497 (1973).

4. Hypothetical model of catecholamine uptake into adrenal medullary vesicles. T.A. Slotkin, Life Sci. 13:675-683 (1973).

5. Reserpine-like effects of harmine on isolated adrenal medullary storage vesicle. H.O. Green and T.A. Slotkin, Mol. Pharmacol. 9:748-755 (1973).

6. Secretion and recovery of catecholamines by the adrenal medulla. N. Kirshner and T.A. Slotkin, Biochem. Pharmacol. in press.

7. Drug-resistant effect of adenine nucleotides and magnesium on catecholamine efflux from isolated adrenal medullary storage vesicles. T.A. Slotkin and H.O. Green, Biochem. Pharmacol., in press.

8. Maturation of the adrenal medulla. III. Practical and theoretical considerations of age-dependent alterations in kinetics of incorporation of catechol- and non-catecholamines. T.A. Slotkin, Biochem. Pharmacol., in press.

9. Adrenal medullary storage vesicles of the spontaneously hypertensive rat. T.A. Slotkin and H.O. Green, Biochem. Pharmacol. in press.

10. Structure-activity relationships for the reserpine-like actions of derivatives of beta-carboline. T.A. Slotkin, Life Sci., in press.

Manuscripts in preparation:

1. Effects of acute administration of reserpine and nicotine on catecholamine stores of the spontaneously hypertensive rat. T.A. Slotkin.

2. Effects of tryptamine and phenethylamine derivatives on catecholamine uptake into isolated rat adrenal medullary vesicles. T.A. Slotkin, Frederic J. Seidler and Martha D. Abou-Donia.

Abstracts:

1. Uptake and storage of amines in isolated adrenal medullary vesicles vesicles of developing rats. T.A. Slotkin, Fed. Proc. 32:783Abs (1973).
2. Maturation of adrenal catecholamine storage vesicles of the rat. T.A. Slotkin, Pharmacologist 15:210 (1973).
3. Adrenal Medullary Vesicles of Hypertensive Rats. T.A. Slotkin and H.O. Green, Clin. Res. 22:13A (1974).
4. Effects of tryptamines on epinephrine uptake into adrenal medullary vesicles. T.A. Slotkin, F.J. Seidler and M.D. Abou-Donia, Pharmacologist, in press.

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